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(71) Applicant (for all designated States except US): **THE GOVERNMENT OF THE UNITED STATES**, as represented by **THE SECRETARY OF HEALTH AND HUMAN SERVICES** [US/US]; National Institute of Health, Office of Technology Transfer, 6011 Executive Boulevard, Suite 325, Rockville, MD 20852-3804 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **LEPPLA, Stephen, H.** [US/US]; 9501 Starmont Road, Bethesda, MD 20817 (US). **AVALLONE, Jennifer** [US/US]; 4 Beehive Lane, Flemington, NJ 08822 (US). **BUGGE, Thomas** [DK/US]; 9802 Bristol Square Lane, Apt. 302, Bethesda, MD 20814 (US). **LIU, Shi-Hui** [CN/US]; 14516 Settlers Landing

Way, Gaithersburg, MD 20878 (US). **OSORIO, Manuel** [US/US]; 4710 Bethesda Avenue #706, Bethesda, MD 20814 (US).

(74) Agents: **FANG, Carol, A.** et al.; Townsend and Townsend and Crew LLP, Two Embarcadero Center, 8th Floor, San Francisco, CA 94111 (US).

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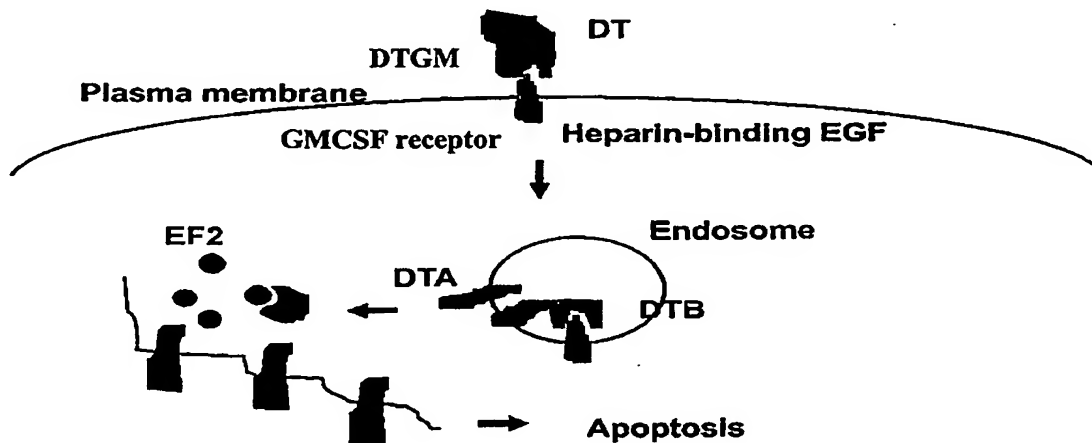
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Declaration under Rule 4.17:

— of inventorship (Rule 4.17(iv)) for US only

[Continued on next page]

(54) Title: **ACTIVATION OF RECOMBINANT DIPHTHERIA TOXIN FUSION PROTEINS BY SPECIFIC PROTEASES HIGHLY EXPRESSED ON THE SURFACE OF TUMOR CELLS**



(57) Abstract: The present invention provides compositions and methods for inhibiting abnormal cell growth. In particular, the invention provides nucleic acids encoding Diphtheria toxin fusion proteins comprising residues 1-388 of Diphtheria toxin, wherein the native furin cleavage site has been substituted for a matrix metalloproteinase or plasminogen activator cleavage site, and a heterologous polypeptide and the polypeptides encoded by such nucleic acids. In addition, the invention provides methods of treating cancer by administering such polypeptides.

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Published:

- with international search report
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER

 IPC 7 C07K14/34 C12N15/62 A61P35/00 A61P35/02 A61K38/19
 C07K14/535

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIU S ET AL: "Targeting of tumor cells by cell surface urokinase plasminogen activator-dependent anthrax toxin" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 276, no. 21, 25 May 2001 (2001-05-25), pages 17976-17984, XP002974279 ISSN: 0021-9258 cited in the application the whole document ----- -/--	1-49

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

28 December 2004

Date of mailing of the international search report

07/01/2005

Name and mailing address of the ISA

 European Patent Office, P.B. 5818 Patentlaan 2
 NL - 2280 HV Rijswijk
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
 Fax. (+31-70) 340-3016

Authorized officer

Sommerfeld, T

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/014306

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LIU SHIHUI ET AL: "Tumor cell-selective cytotoxicity of matrix metalloproteinase-activated anthrax toxin" CANCER RESEARCH, vol. 60, no. 21, 1 November 2000 (2000-11-01), pages 6061-6067, XP002311242 ISSN: 0008-5472 cited in the application the whole document	1-49
Y	FRANKEL ARTHUR E ET AL: "Phase I trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukemia." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH. MAY 2002, vol. 8, no. 5, May 2002 (2002-05), pages 1004-1013, XP002311243 ISSN: 1078-0432 cited in the application the whole document	1-49
Y	BLACK J H ET AL: "Diphtheria toxin-interleukin-3 fusion protein (DT388IL3) prolongs disease-free survival of leukemic immunocompromised mice." LEUKEMIA (BASINGSTOKE), vol. 17, no. 1, January 2003 (2003-01), pages 155-159, XP009041771 ISSN: 0887-6924 cited in the application the whole document	1-49
Y	US 5 677 274 A (ARORA NAVEEN ET AL) 14 October 1997 (1997-10-14) column 10, line 28 - line 51	1-49
Y	WO 01/21656 A (HANSEN BIRKEDAL HENNING ; NETZEL ARNETT SARAH (US); LIU SHI HUI (US);) 29 March 2001 (2001-03-29) cited in the application the whole document	1-49
A	KREITMAN R J: "IMMUNOTOXINS IN CANCER THERAPY" CURRENT OPINION IN IMMUNOLOGY, CURRENT BIOLOGY LTD. LONDON, GB, vol. 11, no. 5, October 1999 (1999-10), pages 570-578, XP001012909 ISSN: 0952-7915	
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US2004/014306

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	<p>ABI-HABIB RALPH J ET AL: "A urokinase-activated recombinant diphtheria toxin targeting the granulocyte-macrophage colony-stimulating factor receptor is selectively cytotoxic to human acute myeloid leukemia blasts." BLOOD. 1 OCT 2004, vol. 104, no. 7, 1 October 2004 (2004-10-01), pages 2143-2148, XP002311244 ISSN: 0006-4971</p> <p>-----</p>	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2004/014306

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 24-48 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US2004/014306

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5677274	A	14-10-1997	US 5591631 A	07-01-1997
			AT 169959 T	15-09-1998
			AU 682500 B2	09-10-1997
			AU 6392294 A	29-08-1994
			CA 2155514 A1	18-08-1994
			DE 69412593 D1	24-09-1998
			DE 69412593 T2	18-02-1999
			EP 0684997 A1	06-12-1995
			ES 2122257 T3	16-12-1998
			WO 9418332 A2	18-08-1994
WO 0121656	A	29-03-2001	AT 283280 T	15-12-2004
			AU 771632 B2	01-04-2004
			AU 2572501 A	24-04-2001
			CA 2385122 A1	29-03-2001
			EP 1214340 A2	19-06-2002
			WO 0121656 A2	29-03-2001